REMARKS/ARGUMENTS

Claims 1-33 are pending in the application.

Claims 7-26 have been cancelled and the cancellation is without prejudice or waiver.

Claims 1-6 and claims 27-33 stand rejected.

Claims 1-6 and claims 27-33 have been previously presented.

The Examiner has acknowledged that the claims under examination are free of prior art.

During recent telephonic interviews, the Examiner raised the issue as to whether a PCA other than with DHFR can be carried out in plants using other reporter fragments. Applicant's would like to point out the following:

"The ability to perform PCA in any cell type, be it bacterial or any eukaryotic cell (including plants) is determined only by whether DNA that encodes for proteins can be introduced into a cell and that under the control of a promoter that can be activated in that cell, the DNA is transcribed into RNA and the RNA translated into protein. This is true for all of the types of cells that applicants' have discussed in our prior applications, including plant cells, tissues and whole plants as described for the generation of transgenic plants with PCA reporters."

Applicant also has shown in its prior US patent No. 6,270,964; how to select, enable and design a reporter molecule i.e., an enzyme reporter and what are the

requirements for successfully performing a PCA using the multitude of reporters which have been exemplified. Once again, for the Examiners' benefit those design requirements are outlined below (See from col. 3 , line 58 to col. 4, line 42:

"One particular strategy for designing a protein complementation assay (PCA) is based on using the following characteristics: 1) A protein or enzyme that is relatively small and monomeric, 2) for which there is a large literature of structural and functional information, 3) for which simple assays exist for the reconstitution of the protein or activity of the enzyme, both in vivo and in vitro, and 4) for which overexpression in eukaryotic and prokaryotic cells has been demonstrated. If these criteria are met, the structure of the enzyme is used to decide the best position in the polypeptide chain to split the gene in two, based on the following criteria: 1) The fragments should result in subdomains of continuous polypeptide; that is, the resulting fragments will not disrupt the subdomain structure of the protein, 2) the catalytic and cofactor binding sites should all be contained in one fragment, and 3) resulting new N- and C-termini should be on the same face of the protein to avoid the need for long peptide linkers and allow for studies of orientation-dependence of protein binding.

It should be understood that the above mentioned criteria do not all need to be satisfied for a proper working of the present invention. It is an advantage that the enzyme be small, preferably between 10-40 kDa. Although monomeric enzymes are preferred, multimeric enzymes can also be envisaged as within the scope of the present invention.

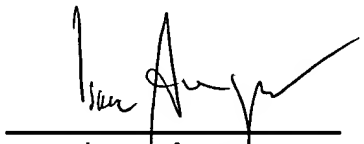
The dimeric protein tyrosinase can be used in the instant assay. The information on the structure of the enzyme provides an additional advantage in designing the PCA, but is not necessary. Indeed, an additional strategy, to develop PCAs is presented, based on a combination of exonuclease digestion-generated protein fragments followed by directed protein evolution in application to the enzyme aminoglycoside kinase. Although the overexpression in prokaryotic cells is preferred it is not a necessity. It will be understood to the skilled artisan that the enzyme catalytic site (of the chosen enzyme) does not absolutely need to be on same molecule.

The '964 patent explains the rationale and criteria for using a particular enzyme in a PCA. FIG. 1 shows a general description of a PCA. The gene for a protein or enzyme is rationally dissected into two or more fragments. Using molecular biology techniques, the chosen fragments are subcloned, and to the 5' ends of each, proteins that either are known or thought to interact are fused. Co-transfection or transformation these DNA constructs into cells is then carried out. Reassembly of the probe protein or enzyme from its fragments is catalyzed by the binding of the test proteins to each other, and reconstitution is observed with some assay. It is crucial to understand that these assays will only work if the fused, interacting proteins catalyze the reassembly of the enzyme. That is, observation of reconstituted enzyme activity must be a measure of the interaction of the fused proteins."

In view of the above remarks, it is respectfully submitted that the claims are now in condition for allowance. Reconsideration and withdrawal of the rejections and objections are requested. The Examiner is invited to contact the undersigned at 703-418-2777 if he feels that further discussion may facilitate the resolution of any outstanding issues.

An early indication of a Notice of Allowance is earnestly solicited.

Respectfully submitted,



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